**Case 1: X-linked agammaglobulinemia**

**Summary:** X linked recessive. Hereditary inability to make antibodies. Subject to recurrent infections of common extracellular baterial pathogens such as h. influenzae, streptococcus pneumoniae, strep pyogenes, and staphylococcus aureus. Major cause of infection are pyogenic bacteria (pus forming due to neutrophils). Normal host response to pyogenic bacteria is production of antibodies that coat the bacteria and fix complement to enhance rapid uptake of bacteria in phagocytes which destroy them. When these infections occur frequently the excessive release of proteolytic enzymes (such as elastase) and from host phagocytes causes damage, especially to the airways of the lung (bronchi lose their elasticity and become site of chronic inflammation-bronchiectasis, can die of chronic lung disease). No problems with intracellular infections (such as viral diseases and intracellular bacteria like tuberculosis) because T cell number and function is normal so there is normal cell mediated responses. No tonsils because they are 80-90% B cells

Gene defect is on long arm of X chromosome Xq22 for BTK which encose the cytoplasmic Bruton’s tyrosine kinase. Found in pre-B cells, B cells, and neutrophils. Is activated at different stages of B cell development by engagement of both the pre-b cell receptor and the B cell receptor. Required to mediate the survival and further differentiation of the progenitor B cells in which successful heavy chain rearrangment has occurred. Pre-B cell receptor uses a signal with BTK. Also required for the survival of mature B cells.

Hereditary deficiency of complement component C3 can clinically mimic XLA because fixation of C3 leads to its cleavage and binding of complement receptors on the surface of phagocytic cells. iC3b binds the most potent complement receptor CR3.

Female could have similar symptoms if any of the nonredundant components of the pre-B cell receptor signaling pathways are deficient. Autosomal recessive for mu heavy chain, lambda 5 surrogate light chain, Iga and b signaling components, LRRC8 truncation, and B cell linked protein BLINK

**Case:** 2 year old boy that was well for first 10 months (was protected by maternal IgG), had pneumonia, otitis media, and erysipelas (strep infection of the skin) in 1 year, gained weight and developed normally but continued to have repeated infections of ears and sinuses and pneumonia, lows serum IgG and IgM, no IgA, ateletasis (collapsed part of lung) and chronic cough caused hospitalization at age 9, no visible tonsils, normal weight counts, elevated monocytes, flow cytometry of T cells was normal but non of peripheral blood lymphocytes bound Ab against B cell marker CD19, T cell proliferation indices were normal mom had 2 brothers that died from pneumonia at age 2, mom had 2 sisters that were well with one having a healthy son and daughter and the other with a healthy daughter, responded to IV doses of gamma globulin

**Treatment:** gamma globulin prepared from human plasma (pooled from 1000 or more donors), fractionated at very cold temperatures by adding increasing amounts of ethanol and obtain 5 Cohn Fractions. Cohn Fraction II is almost pure IgG and is called gamma globulin and is available as a 16% solution although this can’t be giving intravenously because it will aggregate and act like ICs (causes rxn of shaking chills, fever, lower BP) so it is disaggregated with low pH or insoluble proteolytic enzymes and is adminstered IV as 5% solution (Cohn III is beta globulins including IgA and IgM, IV is alpha globulins), fractionatin is followed by purification using anion axchange chromatography and virus removal and inactivtion steps to decrease risk of infection spread. Dose is calculated based on the knowledge that the IgG half life is 21 days due to catabolism, but is 28 days in XLA patients (also minor loses in saliva, tears, gut, other secretions). Distributed into extravascular space (half in blood, half here). Dose administered IV would equilibrate in 24 hrs.

**Explain that only the cells of the B lymphocyte lineage produce immunoglobulins (Ig) and that mature B cells exhibit membrane immunoglobulin. After encountering antigen, mature B cells differentiate into plasma cells that only produce secrete antibody (no more surface Ig).**

**Define how soluble antibodies protect the host in different ways, including the neutralization of toxins and viruses, and by binding to bacteria to cause opsonization (e.g. the Fc portion of IgG flag a bacterium for uptake by macrophages via an Fc receptor).**

**Explain how opsonization by antibody may lead to formation of the membrane attack complex and cell lysis via complement (very few pathogenic bacteria are susceptible to MAC-lysis, but antibody-coated viral-infected cells are easily lysed).**

**For patients that are unable to make antibody, differentiate between their susceptibility to infection by certain extracellular bacterial pathogens (i.e. *Staphylococcus aureus, Streptococcus pneumoniae*, and *Haemophilus influenzae*). Note that these patients do not possess similar susceptibility to most viruses or intracellular bacterial pathogens (i.e. *M. tuberculosis*).**

**Explain the inheritance of X-linked agammaglobulinemia.**

**Explain that X-linked agammaglobulinemia (Bruton’s-stype agammaglobulinemia) is caused by the production of a mutant tyrosine kinase *btk* that is required in B cell development. Also explain that the absence of a functional *btk* kinase prevents the production of a mature B cell.**

**Explain why B cells of carrier females show non-random inactivation of the X chromosome while T cells show a pattern of random inactivation of the X chromosome.**

In somatic cells of females 1 of the X chromones is inactivated so each is normally active in 50% of cells. If the normal X chromosome is inactivated in a BTK carrier in a pre B cell, that cell has no normal BTK gene product and can’t mature. Therefore, all of their B cells have the normal X chromosome active (nonrandom activation). Could use a marker that allows us to distinguish between the 2 X chromosomes and determine whether the B cells exhibit random or nonrandom X inactivation.

**Recognize that the majority of live viral vaccines should not be given to persons with immunodeficiencies.**

Live polio vaccines are made from viruses with a diabling mutating in the gene that allows the virus to enter the motor nerve cells and cause paralysis, given attenuated virus orally and it establishes a harmless infection in the gut. Within 2 weeks the infant makes IgG and IgA antibodies that neutralize the virus and prevent infection from spreading. Immunodeficient people are inacapable of making antibodies and the infection ccan persist. The virus can reacquire the ability to enter nerve cells (neurotropism) and disseminate through the bloodstream to infect SC neurons and cause paralytic poliomyelitis, also echoviruses (enter CNS and cause meningoencephalitis)

**Recognize that B cells bear CD19 and T cells bear CD3, and explain how flow cytometry can be used to distinguish these lymphocyte subpopulations.**

Label cells with fluorescent dyes using dye-coupled antibodies specific for cell surface antigens, label B cell marker CD19, T cell marker CD3

Can cause T cells to proliferate using plant lectins (phytohemagglutinin and concanavalin A) as non specific Ags. Could also give Ags to which host has been previously exposed to. After 72 hrs. tritiated thymidine was added to T cell cultures and becomes incorportated into the DNA of dividing cells, stimulation indices (tritium counts in stimulated cultures divided by number of counts in dimilar cultures not exposed to Ag) were normal showing normal T cell proliferation

**Explain that tonsils are composed largely of B cells.**

**Differentiate the treatment of agammaglobulinemia by either i.v. gammaglobulin (which is specially treated to avoid aggregates which could activate complement) or i.m. gammaglobulin.**

**Case 2: X-linked Hyper-IgM Syndrome**

**Summary:** IgM is the first type of Ig made. Isotype switching occurs in the heavy chain of B cells so that the V regions (specificity) becomes associated with heavy chain constant regions of different isotypes which determine the class of the antibody. Is induced mainly by T cells (although it can happen T cell independently via TLR mediated signaling). T cells are required to initiate B cells response to many Ags via specification interactions involving the T cell surface protein CD40L binding to CD40 on the B cell surface (exceptions are those triggered by some microbial Ags or some Ags with repeating epitopes). Activated T cells secrete cytokines which are required at the initiation of the humoral immune response to drive proliferation and differentiation of naïve B cells and also to later induce class switching (ex: IL 4 and IL 13 to stimulate IgE synthesis).

CD40 also expressed on surfaces of macrophages, DCs, follicular DCs, mast cells, and some epithelial and endothelial cells to trigger initial activation and expansion of Ag specific T cells at the start of an immune response. Evidence for role for CD40-CD40L interaction in the early priming event because absence of either CD40L or CD40 the initial activation and expansion of T cells in response to Ag is greatly reduced.

Gene for CD40L is located on the X chromosome at Xq26. In males with the defect isotype switching doesn’t occur (make IgM and IgD, lack IgG, IgA, or IgE synthesis). Can use demonstrated by isolating T cells and challenge them with soluble fluorescently labeled CD40 to show that activated T cells from patients with CD40L deficiency fail to bind the soluble CD40-Fc. Similar symptoms can be seen in CD40 deficiency (clinically rare, autosomal recessive).

Defects in both humoral and cell mediated immunity. Defects in Ab synthesis results in susceptibility to pyogenic infections (ex: strep pyogenes causing sinusitis) which are resistant to destruction by phagocytic cells unless they are opsonized with Ab and complement. Defects in cellular immunity result in susceptibility to opportunistic infections such as bacteria, viruses, fungi, and protozoa that reside in the body but only cause disease when the immune system is weakened (ex: pneumonia fungus and diarrhea caused by cryptosporidium protozoan, ubiquitous and cause opportunistic infections). Can make IgM response to T cell independent Ags but unable to make Abs against T cell dependent Ags (largely unprotected from many bacteria). Susceptibility to cryptosporidium (can cause persistent inflammation in liver, sclerosis and liver failure) indicates a role for CD40L in the T cell mediated activation of macrophages.

IgD is fine because it is made by alternative splicing.

Patients also have severe neutropenia with a block at the promyelocyte/myelocyte stage of differentiation in the bone marrow (accounts for presence of severe sores and blisters in mouth). Will respond to administration of recombinant G-CSF.

Many patients die in late childhood or adulthood of infections, liver disease, or tumors (lymphomas, neuroectodermal tumors of the gut).

**Case:** 5 yrs. old, hospitalized with severe acute infection of sinuses (ethmoiditis), recurrent sinus infections since 1 yr. old, pneumonia at age 3, b-hemoltic strep cultured from nose and throat, low WBC count, low neutrophils, high monocytes, no antibodies against strep Ag, low IgG and IgA but serum IgM was elevated, lymph node biopsy showed poor organization with absence of secondary follicles and germinal centers, no Ab detection after boosters of typhoid or tetnus. High anti-A and B titers but were of IgM class only (can stimulate B cell response with T cell help), normal peripheral blood lymphocytes upon FACS but all B cells were IgM and IgD, T cells activated in vitro they didn’t bind soluble CD40, no remarkable family history, gamma globulin treatment worked until age 15, cryptosporidium infection, jaundice, elevated liver enzmes, abnormalities of biliary ducts and sclerosing cholangitis (chronic inflammation and fibrosis of bile ducts), died at age 21

**Treatment:** immunoglobulin replacement therapy (gamma globulin), prophylaxis with trimethoprim-sulfamethoxazole (to prevent pneumonia) and protective measures to reduce risk of cryptosporidium infection. Can be cured by hematopoietic cell transplantation (considered when HLA-identical donors are available and when first signs of severe complications manifest)

**Define functions and distributions of different immunoglobulin isotypes (Fig 11.1).**

Complement activation: IgM>IgG3>IgG1>IgG2

Mast cell sensitization: IgE

Neutralization: IgG, IgA, IgM

Opsonization: IgG1>IgG3

Sensitization for killing by NK cells: IgG1, IgG3

Transport across epithelium: IgA dimer>IgM

Transport across placenta: IgG1>IgG3>IgG2

Diffusion into extravascular sites: IgG, IgA monomer

IgG can promote more efficient phagocytosis via a range of Fc receptors on phagocytes and C3 receptors, more efficient at promoting phagocytosis of most bacteria

**Explain that a B lymphocyte in induced by its interaction with T cells to undergo immunoglobulin isotype switching from IgM+IgD to IgG, IgE, or IgA.**

**Describe the interaction with T cells which involves both presentation of antigen by MHC and the interaction of CD40 on the B cell with CD40L (CD40 ligand) on the T cell. In addition, explain that the switching is induced by cytokines secreted by activated T cells.**

**Recognize that either IL-4 or IL-13 induce a switch to IgE (in presence of T cells in co-culture).**

**Explain why lack of expression of CD40L leads to hyper-IgM syndrome with a lack of production of IgA, IgG and IgE antibodies.**

**Explain the locality of the gene for CD40L (i.e. on X chromosome).**

**Describe how CD40-CD40L interaction is required for activation and expansion of T cells when these are being presented antigen by most antigen-presenting cells (e.g. by macrophages and dendritic cells). Explain why lack of this interaction produces a deficiency in the number of T cells.**

**Recognize that CD40L-deficiency causes deficiencies in both cellular (T cell-mediated) and humoral immunity, and that germinal centers do not form within lymph nodes.**

**List *Pneumocystis* pneumonia as a disease associated with deficiencies in T cell-mediated immunity, and the bacteria associated with deficiencies in opsonizing antibody production.**

Opsonization of haemophilus influenzae, streptococcus pneumoniae, streptococcus pyogenes, staphylococcus aureus (resistant to destruction by phagocytes unless they are opsonized)

**Explain that in persons lacking the ability to mount effective T cell interactions with B cells, antibody responses by the B cell are limited to those involving T-independent antigens (these have arrays of repeating epitopes).**

Newborns have difficulty transcribing the CD40L gene (also have immature B cells) and cyclosporin A inhibits CD40L transcription (also IL2 to prevent expansion of T cell clones to suppress all T cell immune response) so both are more susceptible to pyogenic and opportunistic infections

**Case 3: Activation-Induced Cytidine Deaminase (AID) Deficiency**

**Summary:** Cause of hyper IgM syndrome. Increased susceptibility to pyogenic infections only (resembles XLA). Recessive mutation on short arm of chromosome 12 impacting AID gene. Can occur in females.

Process of class switching works most efficiently after the B cell has received signals from activated Th cells (CD40L/CD40 interaction). Uses activation-induced cytidine deaminase to convert cytidine to uridine to trigger DNA breakage. When CD40 and IL4 receptor on B cells are ligated, the AID gene is transcribed and translated to produce the protein. At the same time transcription of cytidine-rich regions at specific isotype-switch sites is induced, which involves the separation of the 2 DNA strands at the sites. AID deaminates cytidine in a single strand of DNA and then converts the cytidine at the switch sites to uridine. Because uridine is not normally present in DNA, it is recognized by the enzyme uracil-DNA glycosylase (UNG), which removed the uracil base from the rest of the nucleotide. The sites are cleaved by a DNA endonucelase to result in single strand DNA breaks. If single strand breaks on opposite DNA strands are near each other, a souble strand DNA break will form at the switch sites. The double strand breaks are then repaired to bring the 2 switch regions together and join the new C region gene to the V region, resulting in class switching.

AID is also used for somatic hypermutation/affinity maturation. AID defects result in accumulation of IgM-positive B cells in lymphoid organs, giving rise to enlarged spleen (splenomegaly) and enlarged lymph nodes (lymphadenopathy).

Mismatch repair mechanism also contributes to class switching downstream of AID and UNG. Complex recognizes U-G mismatches created by AID and with the help of exonuclease1, it excises DNA to create a double strand break if the single strand breaks introduced by AID and UNG on opposite DNA strands are not near enough to form one. Deficency in MMR proteins or Exo1 have reduced isotype switching.

**Case:** 3 yr old girl, adimitted with pneumonia, fever, high respiratory rate, low blood oxygen saturation, enlarged lymph nodes, chest Xray showed diffusion consolidation (whitened areas of the lung due to inflammation indicating pneumonia), past history of pneumonia at 25 months of age and 10 episodes of otitis media, white cell count showed high neutrophils and low lymphocytes, blood culture grew streptococcus pneumnoiae, serum IgM was high while IgA and IgG were low, although she had been vaccinated against tetanus and haemophilus influenzae she had no specific IgG antibodies against them, had blood type A and IgM titer and anti-B antibodies was upper limit of normal while IgG titer was undetectable, normal expression of CD40L on T cells, normal epression of CD40 on B cells, blood cells failed to secrete IgG and IgG after stimulation with anti-CD40 antibody and IL-4 although the cells proliferated normally, reverse transcription-polymerase chain reaction on mRNA reveleaed a point mutation in the AID gene that introduced a stop codon to form a truncated protein

**Explain that activation-induced cytidine deaminase (AID) and uracil-DNA glycosylase (UNG) are both required for immunoglobulin isotype switching, and that defects in either of these enzymes can lead to hyper IgM immunodeficiency.**

**Recognize that the lack of AID in B cells also leads to an inability to undergo somatic hypermutation, which would normally allow affinity maturation of the original IgM specificities.**

**Explain why a patient with AID deficiency does not develop specific IgG antibodies against vaccines, such as tetanus toxoid.**

**Define the autosomal recessive inheritance (i.e., not X-linked) of AID deficiency.**

**Explain why children with AID deficiency, as well as those who have other causes of hyper IgM immunodeficiency, are susceptible to pyogenic bacterial infections, including multiple middle ear infections. (By now you should know the main species of bacteria that cause these problems: check you do!)**

Subject to recurrent infections of common extracellular baterial pathogens such as h. influenzae, streptococcus pneumoniae, streptococcus pyogenes, and staphylococcus aureus. Major cause of infection are pyogenic bacteria (pus forming due to neutrophils). Normal host response to pyogenic bacteria is production of antibodies that coat the bacteria and fix complement to enhance rapid uptake of bacteria in phagocytes which destroy them.

**Differentiate between those immunodeficiencies (caused by defects in AID or UNG) that primarily affect the humoral immune responses and the immunodeficiencies (caused by defects in CD40 or CD40L, which are also hyper IgM deficiencies: see case 11) which cause both T cell-mediated immunodeficiency and deficiencies in humoral immunity.**

CD40L deficiency is a defect of T cells that also results in a failure to activate monocytes/macrophages and dendritic cells via CD40/CD40L interaction. IL-12 is not synthesized. Pulmonary macrophages are inefficient in killing pneumocystis and liver macrophages may be similarly deficient in killing Cryptosporidium leading to chronic inflammation of the bile ducts. Lack of DC activation via CD40 will impair ability to elicit T cell responses and contribute to susceptibility to opportunistic infections. In AID the deficiency is solely in B cells and only results in defects in Ab production.

Can differentiation hyper IgM syndrome caused by intrinsic B cell defect from one caused by CD40L deficiency by measuring IgE synthesis after stimulation of cultured blood lymphocytes with anti CD40 antibody plus IL4. B cells from patients with CD40L defiency will make normal amounts of IgE with the addition of anti-CD40 plus IL4. B cells from patients with AID deficiency, CD40 defiency, or defects in genes downstream of CD40 won’t secrete IgE. Also enlargement of lymph nodes not seen in patients with CD40L or CD40 defiency because they don’t have germinal centers. In contrast, AID deficient patients have normal cell proliferative responses in germinal centers and infections result in enlarged lymph nodes.

**Recognize that germinal centers are still formed in lymph nodes of persons with AID deficiency, as they have normal CD40:CD40L interactions that are needed for germinal center formation.**

**Case 4: Common Variable Immunodeficiency (CVID)**

**Summary:** Immunodeficiency disorder characterized by low serum levels of all switched immunoglobulin types (IgG, A, and E), an imparied ability to produce specific antibodies (even IgM) after exposure to certain Ags, and increased susceptibility to infections of the respiratory and GI tracts (due to low IgG and IgA). Most common primary immunodeficiency that comes to medical attention. Have low percentage of switched memory B cells (detected via the CD27 cell surface marker). Severely deficient in plasma cells and have impaired somatic hypermutation. Hypogammaglobulinemic and the few antibodies that they develop have low affinity. Makes the patient susceptible to chronic and recurrent infections by encapsulated bacteria such as S. pneumoniae and H. influenzae. Clinical course and degree of deficiency vary from patient to patient. Symptoms may appear in early childhood, asolescence, or adulthood but onset of symptoms is usualy 20-30. Recurrent infections of the sinuses, lungs, ears, and GI tract. Air passage in lung may be irreversibly damaged and chronic infections can develop (bronchiectasis). Greater risk of developing autoimmune diseases (ex: hemolytic anemia, pernicious anemia due to AB against intrinsic factor for vit B12 absorption). Increased risk of lymphoma and gastric carcinoma. May develop granulomatous lesions in lungs and skin characterized by the presence of T cells and macrophages.

Most cases are sporadic (not due to an inherited defect). Approx 20% of cases are familial and often associated with autosomal dominent inheritance. Many patients may first present with IgA deficiency and after several years develop full blown CVID (suggests defects in the same genes may underlie both diseases). Heterogenous nature of the disease demonstrated by documented defects in T cells, B cells, and APCs, which suggests that many genes are involved.

Some familial cases may be monogenic disorders, such as a mutation in the gene encoding the TACI receptor on B cell surfaces (seen in 8-10% of patients). Both homozygous and heterzygous mutations have been shown in sporadic and familial cases. Mutations also detected in 1% of people who don’t suffer from recurrent infections (reflects variable penetrance of gene defect). Member of the TNFR superfamily of receptors (along with BAFF-R and BCMA). Normal function of TACI is as a receptor for BAFF (secreted by cells in the follicles of peripheral lymphoid tissue) and APRIL, which mediate isotype switching to IgA and IgG in presence of TGF b or IL10 and to IgE in presence of IL4 after exposure to thymus-independent 2 antigens (ex: bacterial capsular polysaccharides). May have other functions such as promoting plasma cell differentiation and survival. TACI deficient mice have low serum IgA levels and deficient antibody response to immunization with TI-2 Ags such as pneumonia. Mutants also have enlarged spleens and lymph nodes with increasd number of B cells. Also develop autoimmunity.

TACI mutations in patients cause decreased B cell response to stimulation of TLR9 in promoting plasma cell differnetiation and Ig secretion. Impaired ability to secrete IgA and IgG in response to stimulation with APRIL. Impaired ability to produce an Ab response to pneumonccocal vaccine.

TACI might require ligand induced trimerization for signaling. Recruitment of mutant and normal TACI subunits into the complex wil compromise binding of downstream signaling molecules and interfere with signaling (acts as dominant negative). This is why heterozygous patients would have the disease.

Other monogenic mutations in CVID include CD19 deficiency (component of B cell co receptor complex) mutations in ICOS co stimulatory proteins, and possibly a mutation in BAFF-R (mediates effects of BAFF on B cell deelopment and survival, minor role in isotype switching).

**Case:** 40 yr. old woman, recurrent respiratory and GI infections throughout her life, frequent otitis, sinusitis, and tonsillitis as a child, intermittent diarrhea, hospitalized several times for pneumonia and GI infections (including one by the protozoan parasite G. lamblia), diagnosed with thyroid insufficiency when she was 25, enlarged spleen, lower than normal levels of all Ig isotypes, immunized several times with the pneumonccoal vaccine but was unable to produce Abs against all pneumococcal serotypes, normal numbers of B and T cells, no ANA or RF, sister and moth died from GI cancer and non-Hodgkin’s lymphoma, brother diagnosed with CVID, sequencing revealed mutation in TACI gene

**Treatment:** IV immunoglobulin

**Contrast properties of thymus-independent TI-1 and TI-2 antigens, and describe the main immunoglobulin isotype that is produced in response to these antigens.**

TI-1: bacterial LPS, directly induce B cell division, can’t induce affinity maturation or memory B cells

TI-2: bacterial capsular polysaccharides, have highly repetitive structures and stimulate B cells by cross linking B cell receptors, can induce IgM and some class-switching using the TNFR family and BAFF/APRIL

B cell responses to TI Ags provide a prompt and specific IgM response to encapsulated bacteria (pyogenic) such as strep and staph. Ab is produced and coats the bacteria to promote their uptake via phagocystosis.

**Describe how the TNF/TNFR family members BAFF (B cell activating factor belonging to the TNF family) and APRIL (a proliferation-inducing TNF ligand) can replace the requirement for T cell interaction by allowing B cells to undergo some isotype switching in response to TI-2 antigens. Explain that both BAFF and APRIL appear to act via TACI, a receptor on B cells.**

**Explain CVID may be caused in several different ways, including some cases that are linked to a mutation in *TACI*.**

**Explain CVID is characterized by low levels of IgA, IgG, and IgE, a poor ability to mount antibody responses that involve isotype switching, and by significantly enhanced susceptibility to infections, including both respiratory (where IgG and IgA antibodies protect) and gastrointestinal infections (where IgA antibodies are important).**

**List the enhanced risks of developing autoimmune diseases, lymphoma, and gastric carcinoma as major concerns for patients with CVID.**